

What is claimed is:

1. A method for identifying agents which modulate PTEN activity, comprising:

5 a) providing a host cell wherein PTEN is expressed;

b) contacting said host cell with a test agent suspected of modulating PTEN activity;

10 c) assessing said modulation as a function of alterations in activated AKT levels in the presence of said agent.

2. A method as claimed in claim 1, wherein said PTEN is mutated.

15 3. A method as claimed in claim 2, wherein said mutation in PTEN is selected from the group consisting of G129E, G129R, R130M and C124S.

20 4. A method as claimed in claim 2, wherein said PTEN is inactive and said test agent suppresses concomittant AKT activating activity.

25 5. A test agent identified by the method of claim 4.

30 6. A method for gene therapy for the treatment of cancers arising from a mutation in PTEN, comprising administration of a nucleic acid encoding wild type PTEN to a patient in need thereof.

35 7. The method as claimed in claim 6, wherein said PTEN encoding nucleic acid is inserted into a vector having tropism for said cancer cell.

8. The method as claimed in claim 7, wherein said vector is selected from the group consisting of retroviral vectors, adenoviral vectors, shuttle vectors, disabled vaccinia viral vectors, and plasmid vectors.

9. The method as claimed in claim 8, wherein said plasmid vector is encased in an antibody studded liposome, said antibody being immunologically specific for an antigen present on a tumor cell.

10. The method as claimed in claim 9, wherein said tumor cell is a glioma cell and said antigen is the epidermal growth factor receptor.

11. A method as claimed in claim 10, wherein said liposome is a cationic liposome.

12. A method as claimed in claim 7, wherein said vector disposed in a biologically compatible medium is microinjected directly into said cancer cell.

13. A method for identifying agents which modulate PTEN angiogenic activity, comprising:

- a) providing a host cell wherein PTEN is expressed;
- b) contacting said host cell with a test agent suspected of modulating PTEN angiogenic activity;
- c) assessing said modulation as a function of alterations in microvessel density formation in the presence of said agent.

14. A method as claimed in claim 13, wherein said PTEN is mutated.

15. A method as claimed in claim 14, wherein said mutation in PTEN is selected from the group consisting of G129E, G129R, R130M and C124S.

5           16. A method as claimed in claim 13, wherein said PTEN is inactive and said test agent inhibits PTEN mediated angiogenic activity.

10           17. A method as claimed in claim 13, wherein said microvessel density formation determined via immunospecific binding of anti-CD31 antibodies.

15           18. A test agent identified by the method of claim 16.

20           19. A method for identifying agents which modulate PTEN activity, comprising:

- a) providing a host cell wherein PTEN is expressed;
- b) contacting said host cell with a test agent suspected of modulating PTEN activity;
- c) assessing said modulation as a function of alterations in upregulation of TSP-1 in the presence of said agent.

25           20. A method as claimed in claim 19, wherein said PTEN is mutated.

30           21. A method as claimed in claim 20, wherein said mutation in PTEN is selected from the group consisting of G129E, G129R, R130M and C124S.

35           22. A method as claimed in claim 20, wherein said PTEN is inactive and said test agent restores PTEN mediated upregulation of TSP-1.

23. A test agent identified by the method of claim 22.

24. A method for identifying agents which modulate PTEN activity, comprising:

a) providing a host cell wherein PTEN is expressed;

b) contacting said host cell with a test agent suspected of modulating PTEN activity;

c) assessing said modulation as a function of alterations in VEGF levels in the presence of said agent.

25. A method as claimed in claim 24, wherein said PTEN is mutated.

26. A method as claimed in claim 25, wherein said mutation in PTEN is selected from the group consisting of G129E, G129R, R130M and C124S.

27. A method as claimed in claim 26, wherein said PTEN is inactive and said test agent restores regulated PTEN inhibition of VEGF production.

28. A test agent identified by the method of claim 27.

29. A method for identifying agents which modulate PTEN activity, comprising:

a) providing a host cell wherein PTEN is expressed;

b) contacting said host cell with a test agent suspected of modulating PTEN activity;

c) assessing said modulation as a function of alterations in TIMP3 levels in the presence of said agent.

5           30. A method as claimed in claim 29, wherein said PTEN is mutated.

10           31. A method as claimed in claim 30, wherein said mutation in PTEN is selected from the group consisting of G129E, G129R, R130M and C124S.

15           32. A method as claimed in claim 30, wherein said PTEN is inactive and said test agent restores PTEN mediated induction of TIMP3.

          33. A test agent identified by the method of claim 32.

20           34. A method for identifying agents which modulate PTEN activity, comprising:

          a) providing a host cell wherein PTEN is expressed;

          b) contacting said host cell with a test agent suspected of modulating PTEN activity;

25           c) assessing said modulation as a function of alterations in MMP-9 levels in the presence of said agent.

30           35. A method as claimed in claim 36, wherein said PTEN is mutated.

35           36. A method as claimed in claim 35, wherein said mutation in PTEN is selected from the group consisting of G129E, G129R, R130M and C124S.

37. A method as claimed in claim 36, wherein said PTEN is inactive and said test agent restores regulated PTEN suppression of MMP9 activity.

5           38. A test agent identified by the method of claim 37.

39. A method for identifying agents which modulate PTEN activity, comprising:

10           a) providing a host cell wherein PTEN is expressed;

          b) contacting said host cell with a test agent suspected of modulating PTEN activity;

15           c) assessing said modulation as a function of alterations of invasiveness of said cells in the presence of said agent.

20           40. A method as claimed in claim 39, wherein said PTEN is mutated.

          41. A method as claimed in claim 39, wherein said mutation in PTEN is selected from the group consisting of G129E, G129R, R130M and C124S.

25           42. A method as claimed in claim 40, wherein said PTEN is inactive and said test agent restores PTEN mediated inhibition of invasiveness.

30           43. A test agent identified by the method of claim 42.

44. A method for identifying an agent which modulates PTEN phosphatase activity comprising:

35           a) providing an enzymatically active PTEN molecule or peptide fragment in a biological buffer;

b) adding to said buffer a substrate of said PTEN enzyme, said enzymatic action of PTEN on said substrate giving rise to a detectable reaction product;

5 c) contacting said active PTEN molecule or fragment in said biological buffer with an agent suspected of modulating said PTEN phosphatase activity;

d) measuring said PTEN phosphatase activity in the presence and absence of said agent.

10 45. A method as claimed in claim 44, wherein said PTEN is immobilized on a solid support.

15 46. A method as claimed in claim 45, wherein said PTEN activity is assessed in a high throughput format.

47. A method as claimed in claim 44, wherein said PTEN activity is contacted with a plurality of test agents.

20 48. A method as claimed in claim 44, wherein a Malachite green assay is performed to determine said enzymatic activity of PTEN in the presence and absence of said test agent.

25 49. A method for identifying agents having binding affinity for PTEN or peptide fragment thereof, said method comprising:

a) providing a PTEN molecule or peptide fragment in a biological buffer;

30 b) contacting said PTEN molecule or fragment in said biological buffer with a detectably labeled agent suspected of having binding affinity for said PTEN or peptide fragment thereof, such that a detectably labeled complex forms between those agents  
35 having affinity for said PTEN or fragment thereof; and

d) identifying and isolating said detectably labeled complex if present, thereby identifying said agent.

5           50. A method as claimed in claim 49, wherein said PTEN or PTEN peptide fragment is adsorbed to a solid support.

10           51. A method as claimed in claim 50, wherein said method is performed in a high throughput screening format.

15           52. A method as claimed in claim 49, wherein said PTEN or fragment thereof is contacted with a plurality of detectably labeled agents present in a chemical combinatorial library.

20           53. A method as claimed in claim 49, wherein said PTEN or PTEN fragment is expressed on the surface of a phage, and said expressed PTEN or PTEN fragment is contacted with a plurality of detectably labeled agents present in a chemical combinatorial library.

25           54. A method as claimed in claim 49, wherein said PTEN fragment consists essentially of a fragment selected from the group consisting of DLDLTYIYP (SEQ ID NO: 3), YLVLT (SEQ ID NO: 6), YRNNIDD (SEQ ID NO: 8), KGVTTIPSQRRYVYYSYLL (SEQ ID NO: 15), YSYL (SEQ ID NO: 7), YFSPN (SEQ ID NO: 5), RYSDTTDS (SEQ ID NO: 16),  
30           HCKAGKR (SEQ ID NO: 9), DHNPPQ (SEQ ID NO: 10), KGVTTIPSQRRY (SEQ ID NO: 17), HFWVNTFFI (SEQ ID NO: 11), TLTKNDLD---FTKTV (SEQ ID NO: 12), GDKVEF---FTKTV (SEQ ID NO: 13), DKANKDKAN---FTKTV (SEQ ID NO: 14), and HTQITKV (SEQ ID NO: 18).



55. A method as claimed in claim 53, wherein said phage express a PTEN fragment consisting essentially of a fragment selected from the group consisting of DLDLTYIYP (SEQ ID NO: 3), YLVLTL (SEQ ID NO: 6),  
5 YRNNIDD (SEQ ID NO: 8), KGV TIPSQRRYVYYSYLL (SEQ ID NO: 15), YSYL (SEQ ID NO: 7), YFSPN (SEQ ID NO: 5), RYSDTTDS (SEQ ID NO: 16), HCKAGKR (SEQ ID NO: 9), DHNPPQ (SEQ ID NO: 10), KGV TIPSQRRY (SEQ ID NO: 15), HFWVNTFFI (SEQ ID NO: 11), TLTKNDLD--FTKTV (SEQ ID NO:  
10 12), GDIKVEF--FTKTV (SEQ ID NO: 13), DKANKDKAN--FTKTV (SEQ ID NO: 14), and HTQITKV (SEQ ID NO: 18).

56. A method for preventing or inhibiting inflammatory disease in a patient in need thereof,  
15 comprising the administration of an effective amount of a PTEN agonist.

57. A method as claimed in claim 56, wherein said inflammatory disease is selected from the group  
20 consisting of macular degeneration, arthritis, asthma, hay fever, systemic lupus erythrematosis, Crohn's disease, and inflammatory bowel disease.

58. A method as claimed in claim 56, further  
25 comprising the administration of an inhibitor of PI-kinase.

59. A method as claimed in claim 58, wherein said PI-kinase inhibitor is LY294002.

30 60. A method as claimed in claim 56, further comprising the administration of an AKT inhibitor.

61. A method for the treatment of cancer in a patient in need thereof, comprising the administration of an effective amount of a PTEN agonist.

5           62. A method as claimed in claim 61, wherein said PTEN agonist effectively blocks cancer cell metastasis.

63. A method as claimed in claim 61, wherein said PTEN agonist effectively blocks angiogenesis.

10           64. A method as claimed in claim 61, further comprising the administration of at least one additional chemotherapeutic agent.

15           65. A method as claimed in claim 64, wherein said at least one additional chemotherapeutic agent is selected from the group consisting of alkylating agents, antimetabolites, asparaginase, vincristine, vinblastine, anthracyclines, microtubule disrupting agents, taxol, herceptin, and etoposides.

20           66. A method as claimed in claim 61, further comprising the administration of an inhibitor of PI3 kinase.

25           67. A method as claimed in claim 66, wherein said PI-kinase inhibitor is LY294002.

30           68. A method as claimed in claim 61, further comprising the administration of an inhibitor of AKT.

35           69. A method for inhibiting p53 mediated programmed cell death in a patient in need thereof, said method comprising the administration of a PTEN inhibitor.

70. A method for enhancing the chemosensitivity of tumor cells in a patient in need thereof, said method comprising the administration of an PTEN agonist to a patient having a chemoresistant tumor.

71. A method for enhancing the radiosensitivity of a tumor cells in a patient in need thereof, said method comprising the administration of a PTEN agonist to a patient having a radioresistant tumor.

72. A method of gene therapy for the treatment of an inflammatory condition in a patient having a mutation in PTEN, said method comprising delivery of a native PTEN encoding nucleic acid to immune cells of said patient.

73. A method as claimed in claim 72, further comprising the administration of a PI3 kinase inhibitor.

74. A method as claimed in claim 72, wherein said immune cell is selected from the group consisting of mast cells, B cells, T cells, dendritic cells, neutrophils, eosinophils and macrophages.

75. A method for inhibiting immunoreceptor signaling in a patient in need thereof, comprising administration of an effective amount of a PTEN agonist.

76. A method as claimed in claim 75, wherein said immunoreceptor is selected from the group consisting of a T cell receptor, B cell receptor, ITAM-bearing receptor, FcγR, FcεR, and FcαR.

77. A method as claimed in claim 75, wherein said agonist is administered to prevent a condition selected from the group consisting of graft rejection and graft versus host disease.

78. A method for augmenting an immune reaction in a patient in need thereof, comprising administration of an effective amount of an inhibitor of PTEN.

79. A method as claimed in claim 78, wherein said inhibitor is targeted to a cell selected from the group consisting of T cells, B cells, and macrophages.

80. A method for inhibiting aberrant angiogenesis in a patient in need thereof, said method comprising the administration of a PI3 kinase inhibitor.

81. A method as claimed in claim 80, wherein said aberrant angiogenesis is caused by cancer, autoimmune disease, arthritis, systemic lupus erythematosus, inflammatory bowel disease, coronary artery disease, cerebrovascular disease, and atherosclerosis.

82. A method as claimed in claim 80, further comprising the administration of an AKT inhibitor.

83. A method for inhibiting aberrant angiogenesis in a patient in need thereof, said method comprising the administration of an AKT inhibitor.

84. A method as claimed in claim 83, wherein said aberrant angiogenesis is caused by cancer, autoimmune disease, arthritis, systemic lupus erythematosus, inflammatory bowel disease, coronary artery disease, cerebrovascular disease, and atherosclerosis.

85. A method as claimed in claim 83, further comprising the administration of an PI3 kinase inhibitor.

5           86. A method for inhibiting p53 mediated programmed cell death in a patient in need thereof, comprising the targeted administration of a PTEN inhibitor to normal tissues to inhibit stress induced apoptosis thereof, wherein said patient is in need for  
10 such treatment due to a condition selected from the group consisting of myocardial infarction, cerebrovascular insult and gram negative sepsis.

15           87. A method for inhibiting p53 mediated programmed cell death in a patient in need thereof, comprising the targeted administration of a PTEN inhibitor, said PTEN inhibitor inhibiting cellular senescence thereby promoting survival of normal cells.

20           88. A method as claimed in claim 87, wherein said normal cells are selected from the group consisting of brain cells, heart cells, and skin cells.